### Erlotinib

**Cat. No.:** HY-50896  
**CAS No.:** 183321-74-6  
**Molecular Formula:** C₂₂H₂₃N₃O₄  
**Molecular Weight:** 393.44  
**Target:** EGFR; Autophagy  
**Pathway:** JAK/STAT Signaling; Protein Tyrosine Kinase/RTK; Autophagy  
**Storage:**  
- Powder: -20°C for 3 years, 4°C for 2 years  
- In solvent: -80°C for 6 months, -20°C for 1 month

#### SOLVENT & SOLUBILITY

**In Vitro**  
DMSO: ≥ 50 mg/mL (127.08 mM)  
H₂O: < 0.1 mg/mL (insoluble)  
* "≥" means soluble, but saturation unknown.*

<table>
<thead>
<tr>
<th>Preparing Stock Solutions</th>
<th>Solvent Concentration</th>
<th>1 mg</th>
<th>5 mg</th>
<th>10 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 mM</td>
<td>2.5417 mL</td>
<td>12.7084 mL</td>
<td>25.4168 mL</td>
</tr>
<tr>
<td></td>
<td>5 mM</td>
<td>0.5083 mL</td>
<td>2.5417 mL</td>
<td>5.0834 mL</td>
</tr>
<tr>
<td></td>
<td>10 mM</td>
<td>0.2542 mL</td>
<td>1.2708 mL</td>
<td>2.5417 mL</td>
</tr>
</tbody>
</table>

Please refer to the solubility information to select the appropriate solvent.

**In Vivo**  
1. Add each solvent one by one: 0.5% CMC-Na/saline water  
   Solubility: 10 mg/mL (25.42 mM); Suspended solution; Need ultrasonic

2. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline  
   Solubility: ≥ 2 mg/mL (5.08 mM); Clear solution

3. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)  
   Solubility: 2.5 mg/mL (6.35 mM); Suspended solution; Need ultrasonic

4. Add each solvent one by one: 10% DMSO >> 90% corn oil  
   Solubility: ≥ 2.5 mg/mL (6.35 mM); Clear solution

5. Add each solvent one by one: 50% PEG300 >> 50% saline  
   Solubility: 10 mg/mL (25.42 mM); Suspended solution; Need ultrasonic

#### BIOLOGICAL ACTIVITY

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Erlotinib (CP-358774) is a directly acting EGFR tyrosine kinase inhibitor, with an IC\textsubscript{50} of 2 nM for human EGFR. Erlotinib reduces EGFR autophosphorylation in intact tumor cells with an IC\textsubscript{50} of 20 nM. Erlotinib is used for the treatment of non-small cell lung cancer[1].

**IC\textsubscript{50} & Target**

<table>
<thead>
<tr>
<th>Description</th>
<th>EGFR 2 nM (IC\textsubscript{50}, Cell Free Assay)</th>
</tr>
</thead>
</table>

**In Vitro**

Erlotinib (CP-358774) is also a potent inhibitor of the recombinant intracellular (kinase) domain of the EGFR, with an IC\textsubscript{50} of 1 nM. The proliferation of DiFi cells is strongly inhibited by Erlotinib with an IC\textsubscript{50} of 100 nM for an 8-day proliferation assay[1]. The combination of B-DIM and Erlotinib (2 μM) results in a significant inhibition of colony formation in BxPC-3 cells when compared with either agent alone. The combination of B-DIM and Erlotinib (2 μM) results in a significant induction of apoptosis only in BxPC-3 cells when compare with the apoptotic effect of either agent alone[2].

**In Vivo**

Under the experimental conditions, the combination of B-DIM and Erlotinib (50 mg/kg, i.p.) treatment shows significant decrease (P <0.01) in tumor weight compared with untreated control[2]. Erlotinib (20 mg/kg, p.o.) significantly attenuates Cisplatin (CP)-induced body weight (BW) loss when compared to the CP+vehicle (V) rats (P<0.05). Erlotinib treatment significantly improves renal function in CP-N(normal control group, NC) rats. The CP+Erlotinib (E) rats show significant reduction of the levels of Serum creatinine (s-Cr) (P<0.05), blood urea nitrogen (BUN) (P<0.05), urinary N-acetyl-β-D-glucosaminidase (NAG) index (P<0.05), and significant increase of urine volume (UV) (P<0.05) and Cr clearance (Ccr) (P<0.05) compare to the CP+V rats[3].

**PROTOCOL**

**Kinase Assay**[1]

The kinase reaction is performed in 50 μL of 50 mM HEPES (pH 7.3), containing 125 mM NaCl, 24 mM MgCl\textsubscript{2}, 0.1 mM Na\textsubscript{3}VO\textsubscript{4}, 20 μM ATP, 1.6 μg/mL EGF, and 15 ng of EGFR, affinity purified from A431 cell membranes. The compound in DMSO is added to give a final DMSO concentration of 2.5%. Phosphorylation is initiated by addition of ATP and proceeded for 8 mm at room temperature, with constant shaking. The kinase reaction is terminated by aspiration of the reaction mixture and is washed 4 times with wash buffer. Phosphorylated PGT is measured by 25 mim of incubation with 50 μL per well HRP-conjugated PY54 antiphosphotyrosine antibody, diluted to 0.2 μg/mL in blocking buffer (3% BSA and 0.05% Tween 20 in PBS). Antibody is removed by aspiration, and the plate is washed 4 times with wash buffer. The colorimetric signal is developed by addition of TMB Microwell Peroxidase Substrate, 50 μL per well, and stopped by the addition of 0.09 M sulfuric acid, 50 μL per well. Phosphotyrosine is estimated by measurement of absorbance at 450 nm. The signal for controls is typically 0.6-1.2 absorbance units, with essentially no back ground in wells without AlP, EGFR, or POT and is proportional to the time of incubation for 10 mm[1].

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

**Cell Assay**[2]

To test the viability of cells treated with B-DIM, Erlotinib, or the combination, BxPC-3 and MIAPaCa cells are plated (3,000-5,000 per well) in a 96-well plate and incubated overnight at 37°C. A range of concentrations for both B-DIM (10-50 μM) and Erlotinib (1-5 μM) is initially tested. Based on the initial results, the concentration of B-DIM (20 μM) and Erlotinib (2 μM) are chosen for all assays. The effects of B-DIM (20 μM), Erlotinib (2 μM), and the combination on BxPC-3 and MIAPaCa cells are determined by the standard MTT assay after 72 h and is repeated three times. The color intensity is measured by a Tecan microplate fluorometer at 595 nm. DMSO-treated cells are considered to be the untreated control and assigned a value of 100%. In addition to the above assay, we have also done clonogenic assay for assessing the effects of treatment[2].

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**Animal Administration**[2][3]

Female ICR-SCID (6-7 weeks old) mice are randomized into the following treatment groups (n=7): (a) untreated control; (b) only B-DIM (50 mg/kg body weight), intragastric once every day; (c) Erlotinib (50 mg/kg body weight),...
everyday i.p. for 15 days; and (d) B-DIM and Erlotinib, following schedule as for individual treatments. All mice are killed on day 3 following last dose of treatment, and their body weight is determined. One part of the tissue is rapidly frozen in liquid nitrogen and stored at −70°C for future use and the other part is fixed in formalin and processed for paraffin block. H&E staining of fixed tissue section is used to confirm the presence of tumor(s) in each pancreas.

Six-week-old male Sprague-Dawley (SD) rats weighing 180 to 210 g are used. Cisplatin (CP) is freshly prepared in saline at a concentration of 1 mg/mL and then injected intraperitoneally in SD rats (n=28) at a dose of 7 mg/kg on day 0. To investigate the effect of Erlotinib, 28 CP-N rats are divided into two groups. Separate groups (n=14) each of animals are administered with either Erlotinib (20 mg/kg) (CP+E, n=14) or vehicle (CP+V, n=14) daily by oral gavage from day -1 (24 hours prior to the CP injection) to day 3. Vehicle-treated groups receive an equivalent volume of saline. Five male SD rats at the age of 6 weeks are used as a normal control group (NC, n=5). The NC rats are given an equivalent volume of saline daily by oral gavage from day -1 to day 3. At day 4 (96 hours after CP injection), each rat is anesthetized and sacrificed by exsanguination after the cardiac puncture; blood is collected by cardiac puncture and kidneys are collected. Renal tissue is divided; separate portions are snap-frozen in liquid nitrogen or fixed in 2% paraformaldehyde/phosphate-buffered saline (PBS) for later use. All surgery is performed under diethyl ether gas anesthesia, and all efforts are made to minimize suffering.

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REFERENCES


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